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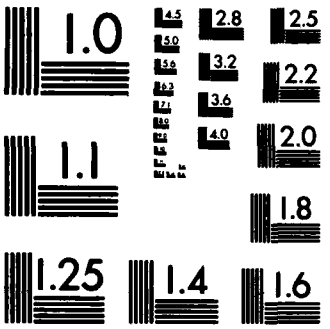

MICROCOPY RESOLUTION TEST CHART
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Basic Studies Related to the Development of a Polyvalent Meningococcal Vaccine

Annual Report

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SUMMARY

This report summarizes results obtained during the first 4 months of a contract to study two aspects of the development of a polyvalent meningococcal vaccine. The first aspect involves Neisseria meningitidis strain 8021 which produces a capsular polysaccharide with the serologic specificities of both the group Y and group W135 capsular polysaccharides. The objective of this contract was to determine, unambiguously, whether strain 8021 produces an immunochemical mosaic molecule in addition to individual molecules with group Y and group W135 specificities. If a mosaic molecule is produced, the ratio of its hexose constituents was to be determined as well as the ratio of the 2 individual molecular species and any lot-to-lot variations in these ratios. In pursuit of these objectives, three separate lots of 8021 capsular polysaccharide have been prepared and purified, using the methods developed at the Walter Reed Army Institute of Research for the preparation of meningococcal capsular polysaccharide vaccines. Antisera to prototype group Y and group W135 strains to be used in the preparation of affinity immunosorbent columns have been prepared in rabbits. The second aspect to be studied involved the nature of the human response to the group 29E meningococcal capsular polysaccharide which has been tested as a vaccine in over 100 human volunteers. In particular, it was planned to determine if the loss of serum bactericidal activity following vaccination with this capsular polysaccharide in some individuals results from preferential induction of circulating IgA, and to confirm and extend preliminary observations that human group 29E capsular polysaccharide antibody binds to the KDO component of the LPS of the Enterobacteriaceae and Neisseriaceae. An immunologic cross-reaction between group 29E capsular polysaccharide and the LPS of a Salmonella minnesota Re mutant was confirmed in both indirect hemagglutination (IHA) and indirect fluorescent antibody (IFA) assays. For the IHA, LPS, extracted and purified from the Re mutant S. minnesota by the chloroform-ether technique, was utilized to sensitize sheep erythrocytes; for the IFA, Re mutant bacteria were used as the substrate and fluorescein conjugated anti-human IgM as the counter-globulin. In both assays, a 3 $\log_2$ reduction in titre of antibody in a post-vaccination serum was produced by absorption with both the homologous Re mutant S. minnesota strain and a prototype group 29E N. meningitidis strain.

FOREWORD

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promolgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences, National Research Council.

I. Introduction.

This report covers the first four months of a proposed twenty-four month project to investigate two discrete questions that have arisen during prosecution of the Walter Reed Army Institute of Research program in meningococcal disease prevention utilizing capsular polysaccharide vaccines. Since the equipment required to complete the project was not received during this report period, progress was limited to those areas which did not require any requested pieces of equipment. The two questions are discrete and will be separately reported.

II. Progress Report.

A. 8021 capsular polysaccharide:

Strain 8021 Neisseria meningitidis produces a capsular polysaccharide with the serological specificities of both the group Y and group W135 on the basis of double diffusion in gel analysis[1]. It was concluded that this strain elaborates both individual molecules of group W135 and group Y immunochemical specificity and a mosaic molecule containing both immunochemical determinants. The data, however, were not unambiguous. It was proposed, therefore, to confirm this interpretation of the gel pattern using sequential affinity immunosorbent chromatography. Using this method, a mosaic molecule could be separated from molecules of individual specificity by its ability to bind to purified antibody against both of the individual determinants which had been immobilized by covalent coupling to cyanogen bromide-activated Sepharose. Antiserum raised in rabbits to prototype strains of each serogroup would be rendered by absorption with the polysaccharide of the opposite serogroup. In addition to the unambiguous demonstration of a mosaic molecule, if any, lot-to-lot variations in the ratio of molecules of individual specificities as well as mosaic molecules were to be determined. This was to be accomplished by the measurement of the ratio of glucose to galactose within molecular species, since both capsular polysaccharides are co-polymers of sialic acid and hexose, either glucose (Y) or galactose (W135).

During the four months covered by this report, three separate lots of strain 8021 capsular polysaccharide have been prepared, and antisera to group Y and group W135 prototype strains have been raised in rabbits. Further progress on this project must await receipt of the requested equipment.

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[1] Brandt, B.L., Pier, G.B., Goroff, D.K., Altieri, P.L., Griffiss, J.McL. Elaboration of both the group W135 and group Y capsular polysaccharides by a single strain of Neisseria meningitidis. J. Gen. Microbiol. 118: 39-43, 1980.

B. Immune response to 29E polysaccharide:

The human immune response to the group 29E capsular polysaccharide is distinctly and unexpectedly different from that to any other bacterial polysaccharide previously studied. Although it is immunogenic as measured by binding antibody assays, the induced antibody does not appear to be bactericidal. More surprisingly, a significant number of individuals experience a reduction in bactericidal titre or remain without serum bactericidal activity despite a brisk binding antibody response following vaccination. Since the bactericidal activity of induced antibody is the functional correlate of its protective capacity, the failure of this capsular polysaccharide to induce bactericidal antibody deserves further attention. Preliminary experiments suggested that this failure might relate to the preferential induction of serum IgA which blocks the bactericidal activity of serum IgG and IgM.

A second unusual aspect of the group 29E capsular polysaccharide is the presence within it of 2-keto,3-deoxy,octulosonic acid (KDO). This unusual carbohydrate molecule is also a part of the K13 Escherischia coli capsular polysaccharide and, most importantly, it is the linking molecule between the lipid and polysaccharide moieties of the LPS of all aerobic Gram-negative bacteria. Preliminary data suggested that a cross-reaction existed between the KDO within the group 29E capsular polysaccharide and that within the lipopolysaccharide of Salmonella minnesota. If this proved true, the 29E capsular polysaccharide might be capable of inducing antibody directed at the lipopolysaccharide of all aerobic Gram-negative bacteria.

The hypotheses to be tested under this contract, then were 1) that group 29E capsular polysaccharide preferentially induces serum IgA in some individuals and both IgA and lytic antibody (IgM and/or IgG) in others; 2) the induced IgA, because of its decreased serum half-life (1/5 that of IgG) declines by six weeks, unblocking induced lytic activity; 3) that whole serum bactericidal activity varies with the ratio of induced IgA to lytic antibody; and 4) that the KDO within the group 29E capsular polysaccharide immunologically cross-reacts with that within the lipopolysaccharide of the Enterobacteriaceae.

During the four months covered by this report, an immunologic cross-reaction between the 29E capsular polysaccharide and enteric LPS has been confirmed using two separate serologic assays.

1. Indirect hemagglutination

LPS was extracted from an Re isogenic mutant of S. minnesota smooth strain SF 1111 by the chloroform-ether technique. After alkali-treatment, the LPS was used to sensitize sheep erythrocytes. Serum from a volunteer immunized two years previously with the group 29E capsular polysaccharide was absorbed thrice with either the homologous S. minnesota Re strain or a prototype group 29E N. meningitidis and tested before and after absorption by indirect hemagglutination. Both the S. minnesota Re and the N. meningitidis group 29E reduced the hemagglutinating titre from 1:16 to <1:2.

2. Indirect fluorescent antibody assay:

This same sera, before and after absorption with the two strains, was also tested in an IgM-specific indirect fluorescent antibody assay. A 3 $\log_2$ reduction in titre of antibody was again effected by absorption with both bacteria.

In order to further explore the nature of this cross-reaction, we have attempted to determine the optimal conditions for indirect fluorescent antibody assays specific for IgM, IgA and IgG. This has, however, proved difficult, and only the IgM-specific assay appears satisfactory at present. Further efforts to optimize conditions for the IFA continue.

III. Conclusions

The first four months of this contract have been primarily used to hire and train a research assistant, to order the necessary equipment and supplies to conduct the experiments and to conduct those preliminary experiments which did not require additional pieces of equipment. Reagents necessary to conduct the strain 8021 capsular polysaccharide project have been prepared; a cross-reaction between group 29E capsular polysaccharide and enteric LPS has been confirmed and indirect fluorescent antibody techniques for further investigating it have been developed, although they are not as yet optimally satisfactory.

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